



Advances in Forensic Biology and DNA Typing

Anna Barbaro and
Amarnath Mishra



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Advances in Forensic Biology and DNA Typing

Advances in Forensic Biology and DNA Typing examines a broad range of forensic DNA applications and topics, based on internationally recognized best practices.

As a contributed volume that includes chapters by experts from around the world, the book covers a broad range of forensic DNA applications and topics. This includes current methods for DNA extraction and typing as well as other technologies and emergent techniques in the field such as Trace and Touch DNA, Forensic DNA Phenotyping (FDP), Forensic Investigative Genetic Genealogy (FIGG), Rapid DNA Biological Fluid Identification by epigenetics, and Pharmacogenomics. The book also explores the development and usage of forensic biology for the analysis of non-human samples and the relevance of DNA databases, management systems and quality certification in forensic.

Key features:

- Highlights sources of DNA (including biological fluids, hair, bones, teeth) detailing how to address the challenges of various sample types, quantities, and environmental factors
- Presents best practices in investigative and collection procedures, as well as evaluative and testing methods, of biological samples
- Addresses both human and non-human DNA analyses and applications for both criminal and wildlife investigations

Advances in Forensic Biology and DNA Typing is a highly illustrated guide that will serve as a useful reference for forensic laboratory professionals, investigators, and students, as well as legal professionals.

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Edited by Anna Barbaro and Amarnath Mishra



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Rapid DNA

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9.1 Introduction

In the world of crime, where every clue matters and every detail counts, a silent witness has emerged. This witness is DNA. The smallest trace of biological material such as a drop of blood, a strand of hair, a smear of saliva, a tooth – holds the key to deciphering the enigma. Teeth are a natural source of stem cells, which have the ability of self-renewal and multidirectional differentiation (Singh et al., 2024). Dental stem cells can be obtained from the periodontal ligament, dental pulp, and apical papilla (Jain et al., 2020). This evidence is capable of identifying a single individual among billions. The power of DNA lies in its unparalleled accuracy. In a situation where a mistake can mean the difference between freedom and imprisonment, life and death, DNA offers a level of certainty that few other forms of evidence can match. It can place a suspect at the scene of a crime, link a series of crimes to a single perpetrator, or even clear the name of someone wrongfully accused (Peterson et al., 2010).

Before, the investigators relied solely on eyewitness accounts or circumstantial evidence such as fingerprints. Fingerprint analysis has been recognized as a trustworthy method for human identification since the 19th century. When a finger contacts a solid object, its secretion leaves a distinctive ridge pattern on the surface (Yadav et al., 2024). Today, the science of DNA analysis like Rapid DNA has made it possible to solve even the most complex cases with astonishing precision and speed. What once took weeks, even months, to unravel can now be done in mere hours, thanks to advancements in DNA technology such as Rapid DNA. As we delve into forensic DNA analysis, we'll explore how this powerful tool has become a beacon of justice, guiding investigators through the murky waters of criminal cases.

The FBI defines Rapid DNA as “the fully automated process of developing a CODIS Core Loci STR profile from a reference sample swab. The swab in-profile out process consists of automated extraction, amplification, separation, detection, and allele calling without human intervention”. The FBI established the Rapid DNA Program Office in 2010 to facilitate its development and integration into law enforcement. FBI Laboratories and federal



Figure 9.1 NetBio Rapid DNA instrument.

agencies such as the National Institute of Standards and Technology (NIST) and the Defence Forensic Science Centre evaluate the instruments. The US government passed the Rapid DNA ACT on 18th August 2017 (Butler, 2011). The law outlines the standards and procedures governing the use of Rapid DNA instrumentations. The FBI mandates requirements for training and quality assurance. The first fully automatic “swab in-profile out” approved by the FBI is NetBio DNA scan Rapid DNA Analysis (Network Biosystem) (Figure 9.1).

9.2 How the Rapid DNA Technology Works

Rapid DNA technology streamlines the process of DNA profiling, enabling the analysis and identification of genetic material in a significantly shorter time compared to traditional methods. It collectively includes a Rapid DNA instrument, the polymerase chain reaction (PCR) short tandem repeat (STR) typing kit/Rapid DNA cartridge, and an expert system software all used to develop and interpret an STR profile. Two currently used primary analysis platforms are being evaluated and employed by forensic laboratories and police agencies. One is the ANDE Rapid DNA Identification System (ANDE Corporation, Longmont, CO, USA), and the other is the Applied Biosystems RapidHIT ID System (Thermo Fisher Scientific, Waltham, MA, USA) (Thong et al., 2015) (Figures 9.2–9.4).



Figure 9.2 ANDE 6E Rapid DNA instrument.

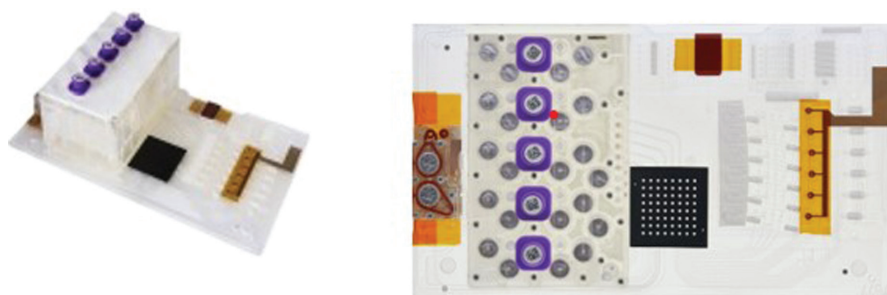


Figure 9.3 Example of a chip used in rapid instruments.

The mechanism is explained below:

1. **Sample Collection:** The process begins with collecting a biological sample, which can include samples of offender, arrestee, detainee, or casework reference sample, it can be a buccal swab which is the most common and non-invasive method, a swab is rubbed to collect epithelial cells, blood samples are used in cases where buccal swabs are not feasible, it is collected using sterile lancet or needle, other samples such as saliva, hair, teeth, bones, muscles tissue, gum, drinking straws can be used. Standardized Swab Kits are used to ensure consistency and reliability it includes the swab, a transport medium, and a secure container to preserve sample integrity. The



Figure 9.4 Oral swab to be used with the ANDE instrument for processing DNA.

sample can be stored at a temperature of -20°C until ready for analysis.

2. **Lysis and DNA Extraction:** The lysis and DNA extraction process is critical for quickly obtaining usable DNA from biological samples. The collected sample is subjected to lysis by a chemical reagent that disrupts the cell membrane, effectively isolating the DNA from other cellular components; enzymes like proteinase K are used to digest proteins and other cellular components. The released DNA is often captured by binding it to a solid phase material, such as silica beads or a membrane, within the cartridge. The bound DNA is then washed with a series of buffers to remove any remaining cellular debris, proteins, and other contaminants. This step ensures that only pure DNA is retained (Figure 9.5).
3. **DNA Quantification and Quality Check:** Before amplification, the extracted DNA undergoes quantification to determine the amount of DNA present. This step ensures that sufficient DNA is available for the subsequent analysis and that the quality is adequate for generating a reliable profile (Figure 9.6).
4. **PCR Amplification:** The core of Rapid DNA technology lies in the PCR, a method used to amplify specific regions of the DNA, known as STRs. STRs are highly variable regions in the human genome, making them ideal for individual identification. Advanced thermal cyclers that rapidly heat and cool the DNA sample are used to facilitate the denaturation, annealing, and extension phases of PCR in



Figure 9.5 Example of DNA Extraction Kit (PrepFiler™ Forensic DNA Extraction Kit).



Figure 9.6 Example of DNA quantification kit (Quantifiler Duo).

minutes rather than hours. The primers used in amplification are pre-designed and pre-loaded into the system to target the STR loci this allows the system to generate a DNA profile that can be directly compared to those in databases like Combined DNA Index System

(CODIS). The system ensures that all STR loci are amplified proportionately, even when DNA quantities vary, to produce a balanced and interpretable DNA profile. The entire process takes place within a sealed, single-use cartridge or chip, which minimizes the risk of contamination and eliminates the need for manual handling of the sample (Figure 9.7).

5. **Separation and Detection:** After amplification, the DNA fragments are separated based on their size using capillary electrophoresis. During this process, the DNA fragments migrate through a capillary tube, with smaller fragments moving faster than larger ones. The fragments are then detected and analyzed using fluorescence detection, which tags the STRs with fluorescent dyes. The resulting fluorescence signals are captured and processed to generate an electropherogram – a visual representation of the DNA profile (Tan & Yiap, 2009).
6. **Profile Generation and Comparison:** It is the process of creating a unique DNA profile from a biological sample after the DNA has been extracted and amplified. The system generates a DNA profile, which consists of a series of STR markers unique to the individual. STR is a sequence of DNA where a short motif (usually two to six base pairs) is repeated multiple times. This profile is then compared to existing profiles in forensic databases, such as CODIS in the United States, to find potential matches (Edwards et al., 1991).



Figure 9.7 Thermocycler GeneAmp® PCR system 9700.

7. **Automated Analysis and Interpretation:** The software analyzes the electropherogram, identifies the STR alleles, and generates a report indicating the presence or absence of a match with existing profiles.
8. **Reporting and Integration with Databases:** The final step involves the reporting of the DNA profile and any matches identified. In law enforcement applications, this information is often integrated with national or international DNA databases, facilitating the rapid identification of suspects or the linking of crimes.

9.3 Application

Law Enforcement and Crime Scene Investigation: It is widely used in law enforcement to quickly generate DNA profiles from crime scene evidence. This allows for the rapid identification of suspects, which can be crucial in solving crimes such as homicides, sexual assaults, and burglaries. The ability to produce results within hours rather than days helps expedite investigations and improves the efficiency of criminal justice processes (Murphy, 2007).

Disaster Victim Identification (DVI): In mass casualty events, such as natural disasters or terrorist attacks, Rapid DNA technology plays a crucial role in identifying victims. By quickly analyzing DNA from remains, authorities can provide closure to families and facilitate the legal and administrative processes that follow such events (Prinz et al., 2007).

Immigration and Border Control: Rapid DNA testing is increasingly used in immigration and border control to verify familial relationships among applicants and to identify individuals at borders. This application helps in preventing human trafficking and in verifying asylum claims where documentation may be lacking (O'Brien, 2022).

Military Operations (International Committee of the Red Cross, 2002): In military contexts, it is used for the identification of soldiers in the field, particularly in cases where conventional identification methods are impractical. This application is critical for both operational security and humanitarian reasons.

Paternity Testing and Familial Matching: It is also used in civil applications such as paternity testing, where results are needed quickly. This is particularly useful in legal cases where time is a critical factor, such as in child custody disputes or inheritance cases (Taitz et al., 2002) (Figures 9.8 and 9.9).



Figure 9.8 RapidHIT ID DNA profiling system.

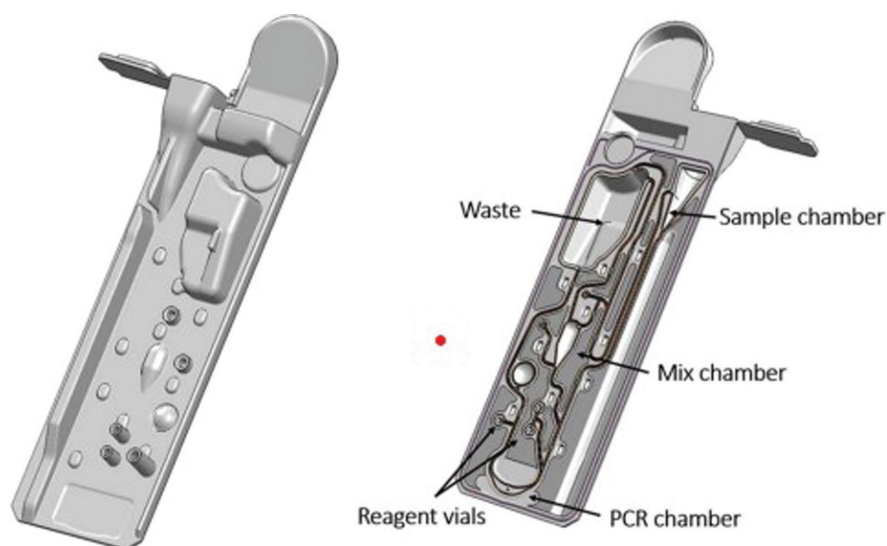


Figure 9.9 Cartridge for RapidHIT including reagent vials.

9.4 Advantages

Speed and Efficiency: Rapid DNA technology allows for the generation of DNA profiles in as little as 90 minutes, significantly reducing the time required for forensic analysis compared to traditional methods, which can take days or even weeks.

On-Site Testing Capability: The portability of Rapid DNA devices enables on-site testing at crime scenes, police stations, or border control locations. This reduces the need to transport samples to a laboratory, which can be time-consuming and risk sample degradation or contamination.

Improved Crime Solving: Rapid DNA technology enhances law enforcement's ability to solve crimes quickly by providing timely leads. Early identification of suspects through DNA can prevent further crimes, assist in clearing innocent individuals, and expedite the justice process.

High Accuracy: Rapid DNA technology has been shown to produce highly accurate and reliable DNA profiles, comparable to those generated by traditional laboratory methods. This accuracy is crucial for its acceptance in legal contexts and its effectiveness in forensic investigations.

Potential for Wide Application: Rapid DNA technology is versatile and can be applied in various fields beyond law enforcement, including disaster victim identification, immigration, and military operations. This broad applicability makes it a valuable tool across multiple sectors.

9.5 Limitations and Challenges

Rapid DNA technology, while revolutionary in its ability to quickly generate DNA profiles, faces several challenges that must be addressed to ensure its effective and ethical use. Ensuring the accuracy and reliability of Rapid DNA results is critical. The technology must consistently produce reliable DNA profiles that meet the standards required for forensic analysis. Rapid DNA technology is highly dependent on the quality of the biological samples. Degraded, mixed, or low-quality samples can result in incomplete or unusable DNA profiles, limiting the technology's effectiveness in real-world applications. Integrating this technology with existing forensic and law enforcement databases poses technical challenges. Compatibility issues, data standardization, and ensuring seamless data transfer are necessary for effective use. The initial costs of acquiring Rapid DNA instruments, along with ongoing maintenance and operation expenses, can be prohibitive for many law enforcement agencies, particularly in smaller or underfunded jurisdictions. There is a risk that the benefits may be unevenly distributed, with well-funded agencies having access to the technology while others do not this could lead to disparities in the quality and speed of forensic investigations across different regions. Regulatory approval such as from the US Food and

Drug Administration (FDA) is required before they can be widely deployed. The approval process can be lengthy and complex, potentially delaying the adoption of the technology. Courts must determine whether Rapid DNA results meet the standards for admissibility as evidence. Legal challenges may arise regarding the technology's reliability, the qualifications of operators, and the maintenance of chain-of-custody protocols.

9.6 Ethical and Legal Considerations

Addressing the ethical and legal considerations in Rapid DNA technology requires a multi-faceted approach that involves the development of policies, frameworks, and practices designed to protect individual rights while enabling the effective use of the technology. Here are strategies to address these considerations:

Data Encryption: Ensure that DNA data is encrypted both in transit and at rest. This protects sensitive genetic information from unauthorized access or breaches.

Access Controls: Limit access to DNA data to authorized personnel only, with strict access controls and auditing mechanisms to monitor and track who accesses the data and for what purpose.

Anonymization: Where possible, anonymize DNA data to protect individual identities, especially in non-criminal contexts.

Clear Communication: Develop consent forms that explain how DNA samples will be collected, used, stored, and shared.

Voluntary Participation: Ensure that participation in DNA testing is voluntary, particularly in contexts outside of criminal investigations (e.g., immigration, health screenings).

Retention Limits: Define specific time frames for retaining DNA data, with automatic deletion or anonymization of data after the retention period expires, unless there is a legitimate reason to retain it longer.

Legislation: Governments should enact and enforce laws that govern the use of Rapid DNA technology.

Regulatory Bodies: Create independent regulatory bodies to oversee the implementation of Rapid DNA technology. These bodies should have the authority to investigate complaints, and audit practices, and ensure compliance with ethical and legal standards.

Non-Discriminatory Practices: Law enforcement agencies should implement policies that prohibit the use of Rapid DNA technology

for racial or ethnic profiling. Training on the ethical use of DNA should be mandatory for all personnel involved.

Transparency: Law enforcement agencies should publicly disclose their use of Rapid DNA technology, including the types of cases in which it is used, success rates, and any incidents of misuse (El-Alfy & Abd El-Hafez, 2012).

Ethical Review Boards: Establish independent ethical review boards to evaluate the implications of new developments in Rapid DNA technology. These boards should regularly review and update guidelines based on technological advancements and societal changes.

Research on Impact: Support research on the social, legal, and ethical impacts of Rapid DNA technology to inform future policy decisions and ensure that the technology is used responsibly.

By addressing these areas, the ethical and legal considerations surrounding Rapid DNA technology can be managed in a way that maximizes its benefits while minimizing potential harms. The goal is to ensure that Rapid DNA technology is used fairly, responsibly, and with respect for individual rights and societal values.

9.7 Case Studies and Real-World Application

Rapid DNA technology has played a role in solving various high-profile cases across different contexts. California Arrestee Cases– In California, Rapid DNA has been used to quickly generate DNA profiles from arrestees. In several instances, this technology has led to the identification of repeat offenders or linking suspects to ongoing investigations within hours of their arrest. Chicago's Gun Crime Reduction Efforts– Rapid DNA technology has been utilized in Chicago to address gun violence. By processing DNA from firearms and other evidence swiftly, investigators have been able to match DNA profiles to known offenders and solve cases more rapidly (de Roo et al., 2023). Hurricane Katrina (2005)– Rapid DNA technology was used to aid in identifying victims in the aftermath of Hurricane Katrina. The technology provided quick results, helping to reunite families and identify individuals who had perished in the disaster (Donkervoort et al., 2008). Thailand's 2004 Tsunami– In the wake of the Indian Ocean tsunami, Rapid DNA was deployed to assist in identifying victims. The technology facilitated faster identification processes amidst the chaos and scale of the disaster (Lessig et al., 2006). Family Verification at US–Mexico Border– Rapid DNA technology has been used at the US–Mexico border to verify the familial relationships

of individuals claiming to be family members. This application has helped to prevent fraudulent claims and ensure the safety and legality of border crossings (Kaye, 2007). Identification in Conflict Zones– Rapid DNA has been employed in conflict zones to identify soldiers and personnel quickly. This has been crucial in accurately identifying individuals in high-stakes and time-sensitive situations (Kaye, 2007). These cases highlight how Rapid DNA technology enhances the efficiency and effectiveness of investigations across various scenarios, from criminal justice to disaster response.

9.8 Future Directions and Advancements

The future of Rapid DNA technology holds exciting potential for further revolutionizing forensic science and other fields. Future developments will focus on improving the sensitivity to handle a broader range of sample types, including degraded or mixed samples this will enhance the reliability and applicability in more challenging forensic scenarios. Innovations in computational algorithms and data analysis will enable more precise interpretation of DNA profiles, reducing the likelihood of errors and improving the accuracy of matches. It will increasingly be integrated with other forensic techniques, such as fingerprint analysis, facial recognition, and digital forensics, to provide a comprehensive approach to solving crimes (Harisha et al., 2023). This will offer more robust and corroborative evidence.

Enhanced data integration and real-time sharing capabilities will allow for seamless communication between Rapid DNA systems and national or international databases, facilitating quicker and more comprehensive investigations(Andrews et al., 1994).The use of Rapid DNA will extend beyond traditional criminal investigations to include applications in public health, security, and personal identification. This could involve rapid genetic screening for health conditions or enhanced biometric identification systems. As technology becomes more affordable and accessible, Rapid DNA systems are expected to be deployed more widely in various sectors, including remote or resource-limited areas, providing valuable capabilities where traditional methods are impractical. The future will necessitate the development of privacy safeguards to address concerns related to consent, data security, and potential misuse. Ongoing dialog and regulation will be essential to balance technological benefits with ethical considerations. Evolving legal frameworks will need to adapt to the capabilities of Rapid DNA technology, ensuring its responsible use while addressing new challenges and setting clear guidelines for evidence handling and data management. Ongoing research will drive innovations in Rapid DNA technology, leading to smaller, more efficient

devices with improved capabilities. This will include advancements in automation, miniaturization, and field deployment. Enhanced portability and ruggedness of devices will enable their use in remote or challenging environments where traditional lab-based analysis would be impractical. In summary, the future of Rapid DNA technology promises to further enhance its role in forensic science and beyond. By advancing accuracy, expanding applications, and addressing ethical and legal concerns, Rapid DNA will continue to be a pivotal tool in solving complex cases and advancing public safety. The ongoing evolution of this technology will shape its impact and effectiveness, making it an essential component of modern investigative and security practices (Murphy, 2015).

9.9 Conclusion

Rapid DNA technology represents a significant advancement in forensic science, offering unprecedented speed and efficiency in the analysis of genetic material. By enabling the rapid processing of DNA samples within hours – this technology has transformed various aspects of criminal investigations, disaster victim identification, and border security. The ability to generate accurate DNA profiles quickly allows for prompt decision-making, enhances investigative efficiency, and supports the timely administration of justice. Despite its advantages, Rapid DNA technology is not without limitations. Challenges related to sample quality, technological constraints, and ethical concerns must be addressed. Privacy issues, data security, and legal implications remain critical to prevent misuse and ensure fair application. Looking ahead, the continued evolution of Rapid DNA technology promises further improvements in speed, accuracy, and applicability. As advancements in DNA analysis and integration with other forensic methods progress, Rapid DNA will likely play an increasingly vital role in solving complex cases and upholding justice. Embracing these innovations while addressing the associated challenges will be key to maximizing the benefits of Rapid DNA and advancing the field of forensic science.

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